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OXYGEN CONSUMPTION AND METABOLIC RATE OF *PAPILIO ZELICAON* PUPAE IN A STATE OF DELAYED ECLOSION

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The metabolic rate of an organism can be estimated from (a) the food consumption, (b) the energy released as heat, or (c) the amount of oxygen used in oxidation processes to obtain the energy. All three methods can be employed, but they are not equally satisfactory. The first method is cumbersome and may give misleading information. The second method, heat production, is technically difficult to carry out, but is nevertheless the most accurate method of determining energy metabolism. The determination of oxygen consumption, the third method, however, is technically easy, gives good results, and, in fact has been used so much that when metabolic rate is mentioned, it usually means rate of oxygen consumption (Schmidt-Nielsen, 1961). The oxygen consumption method is also the most satisfactory method for determining the metabolic rate of pupae.

The function of the pupal stage of metamorphosis is that of structural reorganization. During this phase the metabolic rate falls during the early stages and rises during the latter part of the phase. The oxygen consumption and metabolic rate of the *Papilio zelicaon* pupae, to which moisture had been added, was done in an effort to gain some insight into the reasons for their remaining in the pupal stage for a longer period than is normal; those involved had been in the pupa stage for two years.

There are several factors which influence total metabolism in general; these include temperature, body size, ingestion of food, muscle work, and water. The hypothesis was that the addition of moisture would bring about an increase in the metabolic rate of the pupae thereby causing them to emerge, as it was thought that the reason for the delay was due to insufficient moisture taken in by the pupae during the larval stage.

In the determination of the metabolic rate of the pupae, the exact methods used were taken from those described by Schmidt-Nielsen (1961) in which he employes these methods for the

Fig. 1—Pupae incubation quarters between tests.

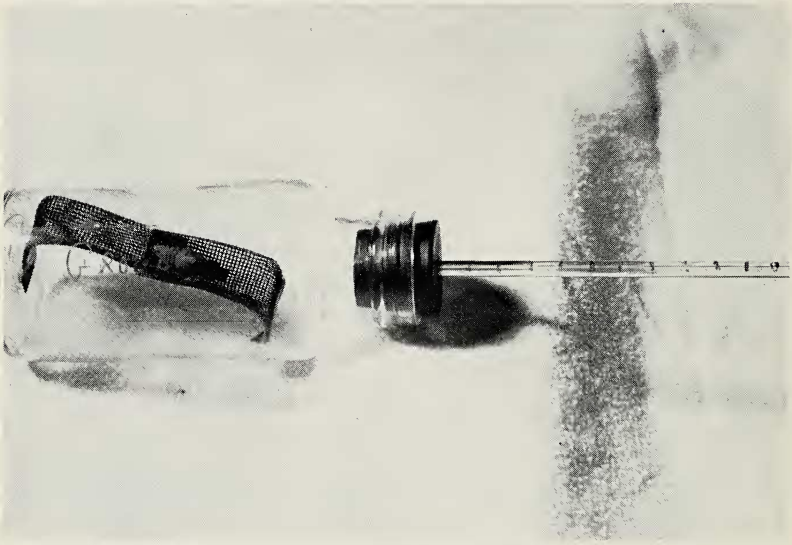


Fig. 2—Apparatus used for determination of oxygen consumption.

oxygen consumption in rats. Information on the methods and selection of experimental subjects was also obtained orally from Mr. Eugene Volz of Sacramento City College.

Information on the effects of moisture upon the eclosion of pupae was obtained from Folsom (1922). He also points out the importance of moisture upon the rate of metabolism in insects. Information was also gained on metabolism in relation to evaporation, and its effect of the eclosion of pupae.

MATERIALS AND METHODS

The pupae used were *Papilio zelicaon* collected as larve from anise plants, their usual food plant, in Sacramento, California during the month of April 1961. They were kept on that diet during their larval stage. The larvae were raised under conditions other than normal in that they were kept indoors and no moisture was given other than that which was gained from eating the food plant.

The pupae were separated into five groups. These groups, which received moisture twice daily were; the positive control group—30 drops, test group A—15 drops, test group B—10 drops, test group C—5 drops, and the negative control which received no moisture. The moisture was applied directly to the pupae with a dropper pipette. During the time they were not being tested the pupae were kept on a table in an open box shown in fig. 1.

For the determination of oxygen consumption, a simple apparatus was used as appears in fig. 2. This equipment included the bottles, one-hole rubber stoppers, copper wire mesh, NaOH crystals and graduated pipettes.

The NaOH crystals, which were used to absorb the CO_2 expired from the pupae, were mixed with hot water to form a syrupy solution and then allowed to cool to room temperature before being poured into the bottles. The bottles were then placed in an insulated campers ice box to keep the temperature constant. A sheet of glass was then placed over the top of the box to ensure these conditions, and the readings were done through the glass. The temperature inside the box was 76 degrees F at all times.

The bottles were placed in a slanting position so that the solution of NaOH was held into one corner of the bottom of the bottle and the copper wire screen was placed in a position so as

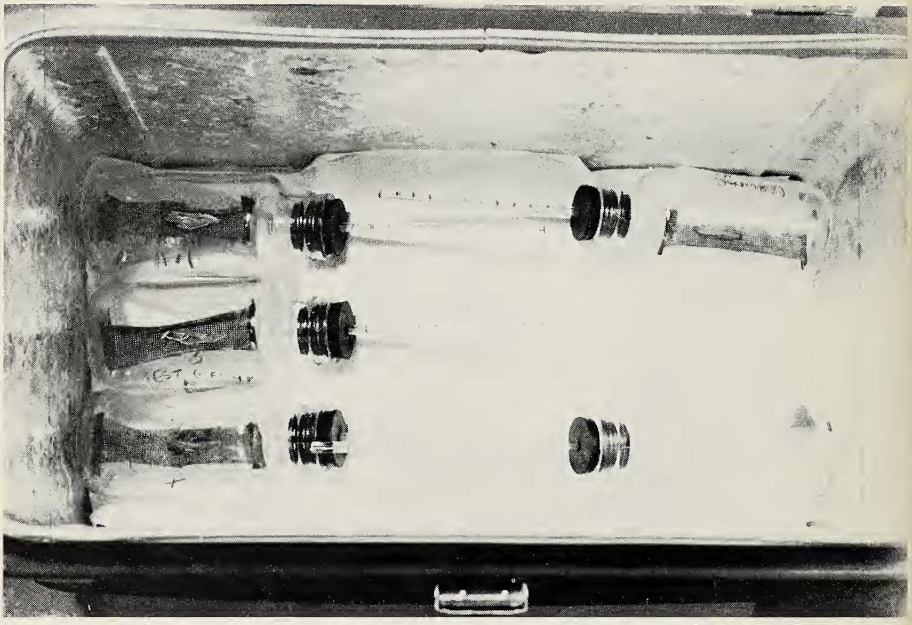
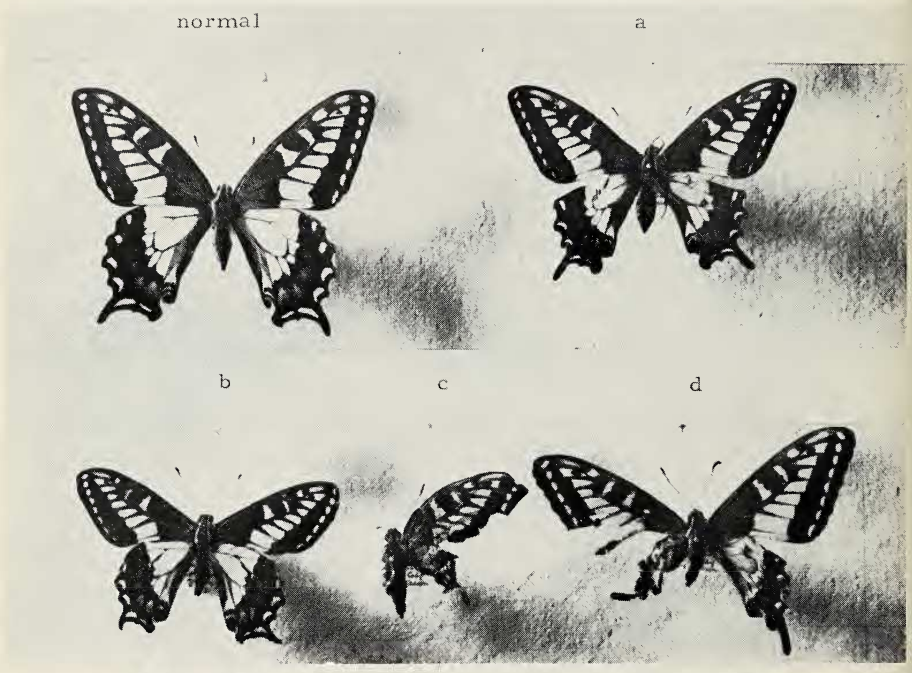


Fig. 3—Set up showing how bottles were arranged in a camper's box during determination of oxygen consumption for respiration rate evaluation.



not to come into contact with the solution. The pupae were placed into the bottles and a period of time allowed to pass before the insertion of the drop of water into the end of the pipette. This was done to allow the temperature of the bottles to return to normal after being handled. A control bottle was also set up to determine if all conditions were correct. A movement in the bottle would have meant the tests were incorrect. To aid in the observation of the drop of water, red dye was added to the water which was inserted into the pipette. The set ups are shown in fig. 3.

Weighings of the pupae were done before and after the tests on an analytical balance, in milligrams.

RESULTS

When the larve which had been raised indoors pupated, the group that remained in the pupal stage long after the others had emerged were assumed to be dead. As the tests to determine the oxygen consumption were made on the pupae it was found that they were all alive and that with the addition of moisture, changes in the metabolic rate were taking place.

In table 1 the oxygen consumption per group for a period of one half hour in micro-liters is shown. Also shown is the percentage of moisture added daily per group. The oxygen consumed increased in proportion to the percentage of moisture added. The data in the table is shown in the graph, fig. 5, and shows the effects of moisture upon the metabolic rate of the groups according to the percentage of moisture added. The groups which received the greatest amount of moisture show a higher rate of oxygen consumption.

Table 2 shows the amount of oxygen and glucose consumed in micro-moles per group during a one half hour time period and also the number of calories released and the Adenosine Triphosphate (ATP) molecules formed during that time. Steady increases are also seen in the groups according to the data obtained.

Fig. 4—Deformed adults shown with a normal female.

a) Group B No. 3—emerged V-15-63. Deformity is seen by holes present in both hindwings.
b) Control group No. 2—emerged III-28-63. Deformity is not clear in photo, but the abdomen was not completely formed and was stuck to the pupal shell.
c) Group B No. 1—emerged IV-29-63. The wings of the left side were not formed. Also note the small left antennae.
d) Group C No. 1—emerged IV-14-63. Left wings were stuck to pupal shell and had to be pulled out. The abdomen is also small and short.

Table 1 - Oxygen consumption per group per one half hour in μl and percentage of water added daily per group during experiments, shown in time in days.

Groups	water added per group daily	Time in Days - μl O_2 consumed 0	7	15	21	25
Control +	saturation	39	157	314	471	785
A	4	39	314	236	157	785
B	1.5	0	314	235	471	393
C	0.75	0	0	0	546	618
Control −	0	0	0	0	157	314

Table 2 - Total group data showing the amount of oxygen and glucose consumed, the number of calories released, and the ATP molecules formed during a one-half hour time period.

Group	μl oxygen consumed per group for .5 hour	volume O_2 consumed and quanity of glucose used up in μ moles	no. of calories released	no. of ATP molecules formed
Control +	352	14.3	9.81	25.9×10^{19}
A	306	12.5	8.62	22.5×10^{19}
B	283	11.1	7.65	20.5×10^{19}
C	233	9.5	6.56	17.5×10^{19}
Control −	94	3.8	3.78	10.3×10^{19}

The weighings of the pupa taken during the experiment are shown in Table 3, and show the weight loss per pupa as the metabolic rate increased due to the added moisture.

Five of the pupae emerged during the experiment, four of which are shown with a normal specimen in fig. 4. The fifth pupa was so deformed it could not completely break through the pupal shell, as the wings had not formed and were still attached to the shell. This pupa was from the positive control group. A sixth pupa, test group C No. 2, was dissected as it had shown a weight loss way below the others which had emerged and it was assumed that the pupa was parasited. When dissected, only the wings and part of the thorax remained, the rest was eaten away leaving an empty shell.

DISCUSSION

With the addition of moisture to the pupae it was shown that the rate of oxygen consumption increased according to the percentage of moisture added. This rise did not seem to be on a straight line however, with the group curves rising and falling, fig. 4. A reference from Rogers (1927) could perhaps give the reason for this fluctuation. "In the pupal stage the respiratory quotient (of *Bombyx mori*) has a value of 0.6 or less. The glucose present in the body is not all used in respiration but may be utilized in building imaginal tissue. Glucose is synthesized at first from protein but during the latter part of the pupal stage apparently from fat. The respiration curve falls during the early pupal stage and rises during the latter part of that stage". The oxygen consumption of the pupae was much higher as they approached the time of eclosion. The relationship of the loss of weight by the pupae and the time of eclosion was also shown. The pupae began to emerge when the body weight was down in the area of 670-770 mg. as shown in Table 3.

That the addition of moisture to the pupa brought about a rise in metabolism which resulted in the eclosion of the adult butterfly, which is also the hypothesis, would seem to be true. The emergence of a pupa from the negative control group, which received no moisture, tends to disprove the hypothesis. The thought that the temperature might have had a part in this was brought to mind although the pupae had been kept at room temperature for the two year period. Also to be considered was the humidity and perhaps the amount of moisture and nutrition which had been taken in by the pupa during the larve stage. That temperature is important in the eclosion of pupae is known and Biliotti (1953) has shown with various temperatures the pupae in a state of delayed eclosion were made to emerge .

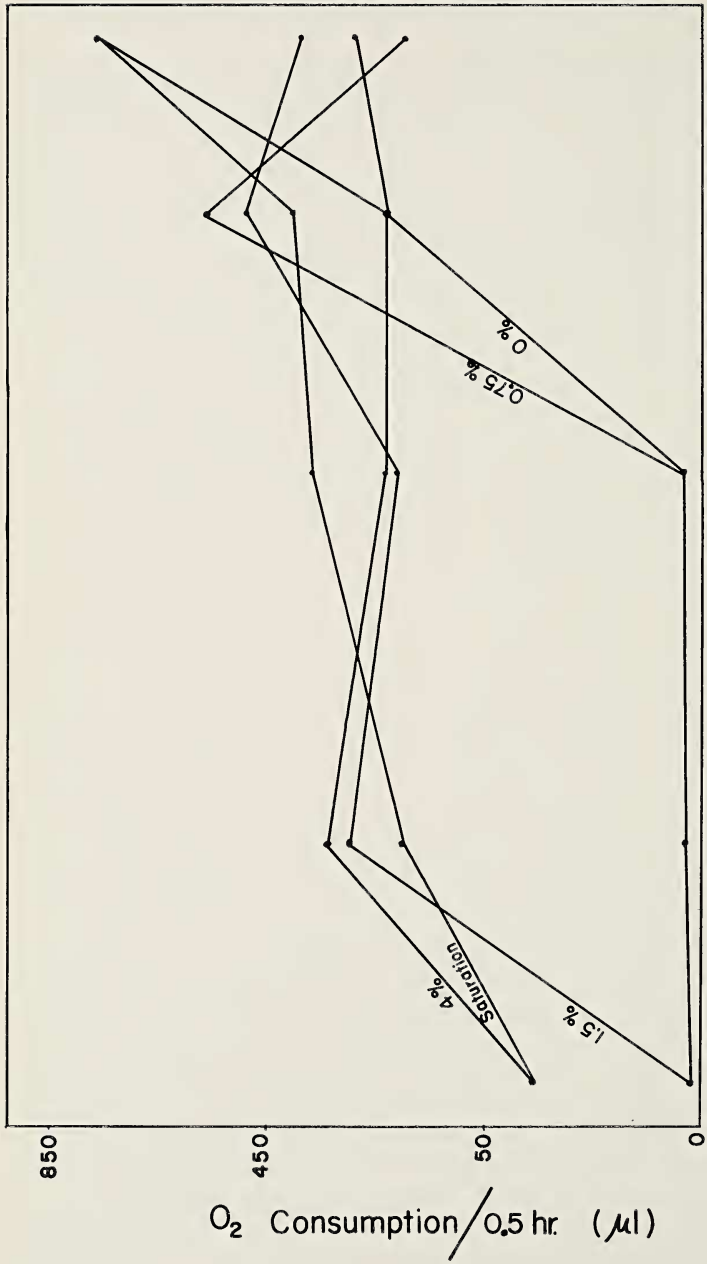


Fig. 5—Effects of moisture upon the metabolic rate per group.

Table 3. Weight of pupae before and after tests, showing the loss in weight in milligrams as the metabolic rate increased.

Pupa Control	Time in Days				
Group +	0	9	17	23	25
1	860	820	800	700	670
2	870	850	800	800	800
3	940	900	900	900	870
Group A					
1	1060	1000	1000	950	950
2	1020	900	870	870	870
3	1000	1000	890	850	850
Group B					
1	900	870	760		
2	850	800	800	750	750
3	1060	870	800	800	770
Group C					
1	900	850			
2	1000	550	350	300	250
3	900	900	800	750	750
Control —					
1	780				
2	860	850	800	800	800

Eclosion of pupa

Moisture ranks with temperature as a highly essential condition of existence and plays an important part in growth. That moisture is important to the eclosion of pupae is stated by Folsom (1922) "Moisture frequently determines the time of eclosion, or the emergence of an insect from the pupa. Hessian flies do do emerge from the puparia in dry weather, but issue in abundance after rainfall in the proper season. When bred indoors, the flies do not emerge from dry soil, even though the temperature be favourable, but emerge shortly when the soil is moistened". He also states that when raising moths from pupae that the pupae must have a certain amount of moisture or they will dry out and die. In comparing this with the results of the experiment it would seem to support the hypothesis.

That the adult butterflies were deformed when they emerged may be the reason they did not emerge at the normal time. It can only be assumed that during the larval stage they did not receive adequate nutrition and moisture to allow them to develop normally and that with the moisture applied they were able to complete the pupal cycle but that the delayed eclosion caused the deformities. Since only five out of the groups have emerged at the time of this writing it is too soon to reach a conclusion on this.

CONCLUSION

The results of the tests made on the oxygen consumption and metabolic rate of the pupae have shown that with the addition of moisture, the metabolic rate of the groups of pupae was raised, and resulted in the eclosion of the adult butterfly. It was also shown that the amount of oxygen consumed was appreciably influenced by the percentage of water added per group. The group receiving the most moisture consumed the most oxygen; this corresponds to a greater number of calories released and the formation of the greater number of ATP molecules.

"The author wishes to express her appreciation to her husband,Noel La Due, for his photography and assistance with the study. Appreciation is also expressed to Mr. Jacques Ricard of Sacramento City College for his help and advice during the entire study."

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